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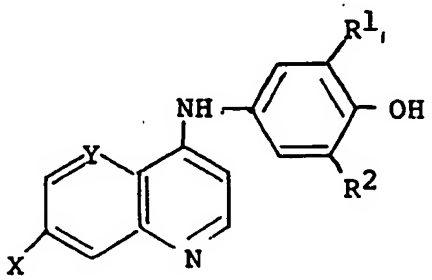

(I)

(57) Abstract

Compounds of general formula (I) have antimalarial activity, wherein X represents a halogen or hal gen-substituted alkyl group; Y represents CH or N; and R^1 and R^2, which may be the same or different, represent hydrogen, aminoalkyl, mono- or di-alkylaminoalkyl, mono- or di-(substituted alkyl)aminoalkyl, mono- or di-(cycloalkyl)aminoalkyl, mono- or di-(aryl)aminoalkyl, mono- or di-(substituted aryl)aminoalkyl, or alkyl substituted by a nitrogen-containing heterocyclic or mono- or di-alkyl-substituted heterocyclic group; provided that R^1 and R^2 are not both hydrogen; and that R^2 is not diethylaminomethyl when X is chloro, Y is CH and R^1 is hydrogen; or when X is bromo, Y is N and R^1 is hydrogen. Processes for the preparation of these compounds are also disclosed.

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"ANTIMALARIAL COMPOUNDS"

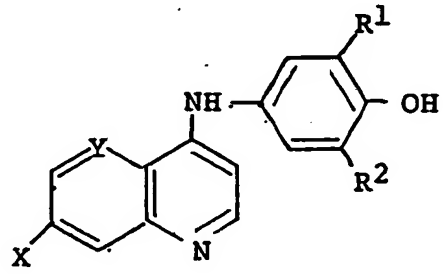
The present invention relates generally to having compounds having antimalarial activity, to processes for the preparation thereof, to pharmaceutical compositions containing these compounds and to methods of treatment using the compounds or compositions.

In particular, the present invention relates to a class of mono- and di-Mannich bases, derived from 4-(7'- substituted -1', 5'- naphthyridin-4'-ylamino) phenols and 4-(7'- substituted- quinolin -4'-ylamino) phenols which have been found to exhibit antimalarial activity against a number of species of Plasmodia, particularly the principal human malarial parasite Plasmodium falciparum.

In work leading to the present invention, the inventors have synthesised and tested a series of 1,8-naphthyridines, N^4-substituted 2-methoxy(and 2-hydroxy)-1,5-naphthyridin-4-amines and N^4-substituted 7-bromo- 1,5-naphthyridin-4-amines for antimalarial activity against Plasmodium vinckei vinckei in mice (Barlin, G.B., and Tan, W.-L., Aust.J.Chem., 1984, 37, 1065; 1984, 37, 2469; and 1985, 38, 459). It has now been found that a series

of mono- and di-Mannich bases derived from 4-(7'-substituted-1',5'-naphthyridin-4'- ylamino) phenols and 4-(7'-substituted-quinolin-4'-ylamino) phenols are able to produce complete cures in the P.vinckei vinckei - mouse model, and also have significant activity against P.falciparum when compared with the activities of the established antimalarial drugs chloroquine, mefloquine, and amodiaquine, including activity against both chloroquine-sensitive and chloroquine-resistant isolates of P.falciparum.

According to a first aspect of the present invention, there are provided compounds of the general formula (I):



I

wherein X represents a halogen or halogen-substituted alkyl group;
Y represents CH or N; and
R^1 and R^2, which may be the same or different, represent hydrogen, aminoalkyl, mono- or di-alkylaminoalkyl, mono- or di-(substituted alkyl)aminoalkyl, mono- or di-(cycloalkyl)aminoalkyl, mono- or di-(aryl)aminoalkyl, mono- or di-(substituted aryl)aminoalkyl, or alkyl substituted by a nitrogen-containing heterocyclic or mono- or di-alkyl-substituted heterocyclic group;

provided that R^1 and R^2 are not both hydr gen; and that R^2 is not diethylaminomethyl when X is chloro, Y is CH and R^1 is hydrogen; or when X is bromo, Y is N and R^1 is hydrogen.

Preferred subgroups of compounds within the general formula I are compounds in which X represents halogen and Y represents N, and compounds in which X represents halogen substituted alkyl and Y represents CH.

Preferably X represents a bromo, chloro or fluoro group, or a trifluoromethyl group.

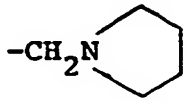
Preferably also, R^1 and/or R^2 represent aminomethyl, mono- or di-alkylaminomethyl, mono- or di-(substituted alkyl)aminomethyl, mono- or di-(cycloalkyl)aminomethyl, mono- or di-(aryl)aminomethyl, mono- or di-(substituted aryl)aminomethyl, or methyl substituted by a nitrogen-containing heterocyclic or mono- or di-alkyl substituted heterocyclic group.

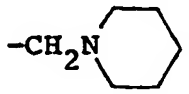
Particular preferred groups are represented by R^1 and/or R^2 are:

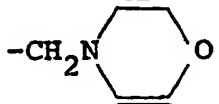
$-CH_2NMe_2$

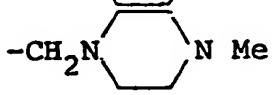
$-CH_2NEt_2$

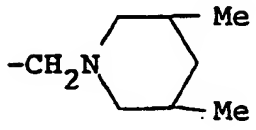
$-CH_2NPr_2$

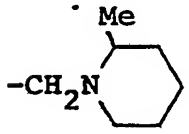






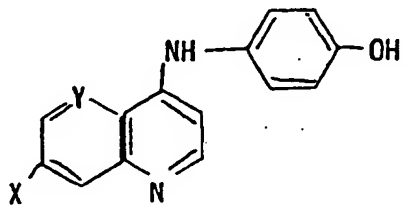






Particularly preferred compounds of this invention are the di-Mannich bases, i.e. compounds in which both R^1 and R^2 are other than hydrogen.

In another aspect, this invention provides a process for the preparation of compounds of the general formula (I), which comprises reaction of a compound of the general formula (II):



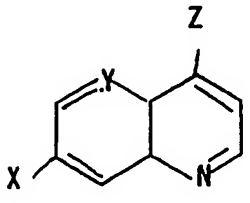
II

in which X and Y are as described above, with an appropriate amine or nitrogen-containing heterocyclic compound in the presence of formaldehyde.

Preferably, the compound of general formula II is refluxed with the appropriate amine or heterocyclic compound in ethanolic formalin. Where a moderate amount of the amine or heterocyclic compound is used, the mono-Mannich base is prepared. Excess amounts of the amine or heterocyclic compound lead to preparation of the di-Mannich bases.

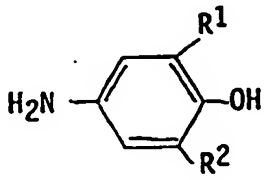
The compounds of the general formula II in which X is bromo and Y is N may be prepared from 3-bromo-8-chloro-1,5-naphthyridine (Barlin, G.B., and Tan, W.-L., Aust.J.Chem.1985, 38, 459) by heating with aqueous methanolic p-aminophenol hydrochloride. Similarly, the compound of the general formula II in which X is trifluoromethyl and Y is CH may be prepared from 4-chloro-7-trifluoromethyl quinoline (Snyder, H.R., et.al., J.Am.Chem.Soc., 1947, 69, 371) by refluxing with aqueous methanolic p-aminophenol hydrochloride. Other compounds of the general formula II may be prepared in an analogous manner.

Alternatively, the compounds of the general formula I may be prepared by reaction of a compound of general formula III:



III

in which X and Y are as described above, and Z represents halogen, with an aminophenol of the general formula IV:



IV

in which R^1 and R^2 are as described above.

Preferably, this reaction is performed under reflux in aqueous methanol.

The compounds of the present invention have exhibited antimalarial activity when screened by in vivo evaluation for activity against P.vinckei vinckei in mice following preliminary examination of each compound for toxicity and for safe dosage levels prior to the antimalarial studies. In addition, in subsequent in vitro tests against both chloroquine-sensitive and chloroquine-resistant strains of P.falciparum a number of compounds of this invention have been more effective than chloroquine, against the sensitive strain, and approximately as effective as mefloquine and amodiaquine against the resistant strain. Certain compounds of this invention have also shown activity in vivo against P.berghei in mice.

Accordingly, the present invention also provides a pharmaceutical composition for use in treatment of malaria in an animal, including a human, which comprises an effective amount of a compound of

the general formula I, together with a pharmaceutically acceptable carrier or diluent therefor.

In another aspect, there is provided a method of treating malaria in an animal, including a human, which comprises administering to the animal an effective amount of a compound of the general formula I.

Further details of preferred compounds in accordance with the present invention, and of the process for the preparation thereof, are given in the following Examples. In these Examples, solids and oils for analysis were dried at 100°C/20mm Hg unless otherwise specified, and melting points were taken in Pyrex capillaries. All structures were confirmed by n.m.r. spectral analysis.

EXAMPLE 1

(a) 4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)phenol (1a)

3-Bromo-8-chloro-1,5-naphthyridine (2.0g), p-aminophenol hydrochloride (1.2g), water (40.0ml) and methanol (20.0ml) were heated with stirring in an oil bath at 100° for 2h. The methanol was then evaporated under reduced pressure and the remaining aqueous solution was adjusted to pH 8 with ammonium hydroxide. The yellow precipitate which formed was filtered off, washed with water, dried and recrystallized from methanol to give 4-(7'-bromo-1',5'-naphthyridin-4'-ylamino)phenol (2.5g), m.p. 245-247°. (Found: C,53.6; H,3.2; N,13.2. $C_{14}H_{10}BrN_3O$ requires C,53.2; H,3.2; N,13.3%).

(b) 4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2-dimethylaminomethylphenol (TN83).

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)phenol (0.5g), formalin (2.0ml; 36%), and ethanolic dimethylamine (1.0ml; 33%) in ethanol (10.0ml) were refluxed with stirring for 20h. The reaction mixture was evaporated under reduced pressure and the residue purified by t.l.c. (silica; methanol) to give an oil (0.25g).

This oil was treated with ethanolic hydrogen bromide and the product recrystallized from ethanol to give yellow crystals of 4-(7'-bromo-1',5'-naphthyridin-4'-ylamino)-2-dimethylaminomethylphenol dihydrobromide (0.3g), m.p. >305° (dec.). (Found: C, 38.4; H,3.7; N,10.4. $C_{17}H_{19}Br_3N_4$ requires C, 38.2; H, 3.6; N, 10.5%).

EXAMPLE 2

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2-(N,N-dipropylaminomethyl)phenol (TN 84).

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)phenol (0.5g), dipropylamine (0.48g), formalin (2.0ml; 36%) and ethanol (10.0ml) were refluxed with stirring for 20h and the mixture worked up as described above. The produce was purified by t.l.c. (alumina; chloroform then silica; ethanol) and recrystallized from light petroleum (b.p. 60-80°) to give yellow crystals of 4-(7'- bromo-1',5'-naphthyridin-4'-ylamino)-2-(N,N-dipropylaminomethyl)phenol (0.15g), m.p. 138-139°. (Found: C, 58.7; H, 5.9; N, 13.2. $C_{21}H_{25}BrN_4O$ requires C, 58.7; H, 5.9; N, 13.1%).

EXAMPLE 3

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2-pyrrolidin-1"-ylmethylphenol (TN 87).

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)phenol (0.5g), pyrrolidine (0.15g), formalin (2.0ml; 36%) and ethanol (10.0ml) were refluxed with stirring for 10h and worked up as described above. The crude produce was purified by t.l.c. (silica; methanol) and the oil (0.27g) was treated with ethanolic hydrogen bromide and the solid recrystallized from ethanol to give 4-(7'- bromo-1',5'-naphthyridin-4'-ylamino)-2-pyrrolidin-1"-ylmethylphenol dihydrobromide, m.p. 318° (dec.). (Found: C, 41.0; H, 3.8; Br, 42.8; N, 9.6. $C_{19}H_{21}Br_3N_4O$ requires C, 40.7; H, 3.8; Br, 42.7; N, 10.0%).

EXAMPLE 4

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(dimethylaminomethyl)phenol (TN 78).

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)phenol (0.5g), formalin (10ml; 36%) and ethanolic dimethylamine (30ml; 33%) were refluxed with stirring for 20h. The product was isolated as described above and purified by t.l.c. (alumina; chloroform) to give 4-(7'-bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(dimethylaminomethyl)phenol (0.5g) as a yellow oil. (Found: C, 55.4; H, 5.8; N, 16.0. $C_{20}H_{24}BrN_5O$ requires C, 55.8; H, 5.6; N, 16.3%)

The tripicrate was prepared in and recrystallized from, ethanol. It had m.p. 152-153°. (Found: C,41.0; H, 3.1; N, 17.1. $C_{20}H_{24}BrN_5O.3(C_6H_3N_3O_7)$ requires C, 40.8; H, 3.0; N, 17.5%).

EXAMPLE 5

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(diethylaminomethyl)phenol (TN 77).

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)phenol (0.5g), formalin (5.0ml; 36%), diethylamine (5.0ml) and ethanol (10.0ml) were refluxed with stirring for 20h. The product was purified by t.l.c. (silica; methanol) to give a yellow oil (0.7g).

A sample of this oil with ethanolic picric acid gave a yellow precipitate which was recrystallized from ethanol to yield 4-(7'-bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis(diethylaminomethyl)phenol tripicrate, m.p. 191-193°. (Found: C, 42.6; H, 3.4; N, 16.4. $C_{24}H_{32}BrN_5O.3(C_6H_3N_3O_7)$ requires C, 43.0; H, 3.5; N, 16.7%).

EXAMPLE 6

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(N,N-dipropylaminomethyl)phenol (TN 79).

4-(7'-bromo-1',5'-naphthyridin-4'-ylamino)phenyl (0.5g), formalin (5.0ml; 36%), dipropylamine (5.0ml) and ethanol (10.0ml) were refluxed with stirring for 20h. The 4-(7'-bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis(N,N-dipropyl-aminomethyl)phenol (0.5g) was isolated as a yellow oil after t.l.c. (alumina; ethanol). (Found: C, 61.8; H, 7.5; N, 12.7. $C_{28}H_{40}BrN_5O$ requires C, 62.0; H, 7.4; N, 12.9%).

The tripicrate was prepared in, and recrystallized from, ethanol. It had m.p. 176-178°. (Found: C, 45.0, H, 4.0; N, 15.7. $C_{28}H_{40}BrN_5O.3(C_6H_3N_3O_7)$ requires C, 44.9; H, 4.0; N, 15.9%).

EXAMPLE 7

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(pyrrolidin-1"-ylmethyl)phenol (TN 80).

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)phenol (0.5g), formalin (5.0ml; 36%), pyrrolidine (5.0ml) and ethanol (10.0ml) were refluxed with stirring for 20h. Excess reagents were distilled and the product purified by t.l.c. (silica; methanol) to give as a yellow oil 4-(7'-bromo-1,-5'-naphthyridin-4'-ylamino)-2,6-bis(pyrrolidin- 1"-ylmethyl)phenol (0.64g). (Found: C, 59.9; H, 6.2; N, 14.0. $C_{24}H_{28}BrN_5O$ requires C, 59.8; H, 5.9; N, 14.5%).

EXAMPLE 8

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(piperidin-1"-ylmethyl)phenol (TN 81).

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)phenol (0.5g), formalin (5.0ml; 36%), piperidine (5.0ml) and ethanol (10.0ml) were refluxed with stirring for 20h. Workup was as described above to give 4-(7'-bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(piperidin-1"-ylmethyl)phenol (0.6g) as a yellow oil which became a semi-solid. (Found: C, 61.5; H, 6.5; N, 13.5. $C_{26}H_{32}BrN_5O$ requires C, 61.2; H, 6.3; N, 13.7%).

EXAMPLE 9

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(morpholin-4"-ylmethyl)phenol (TN 82)

4-(7'-Bromo-1,5'-naphthyridin-4'-ylamino)phenol (0.5g), formalin (5.0ml; 36%), morpholine (5.0ml) and ethanol (10.0ml) were refluxed as described above. The product was purified by t.l.c. (alumina;

chloroform) to give as a yellow oil 4-(7'-bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis(morpholin-4"-ylmethyl)phenol (0.56g). Found: C, 56.4; H, 5.7; N, 13.2. $C_{24}H_{28}BrN_5O_3$ requires C, 56.0; H, 5.5; N, 13.6%).

The dipicrate was prepared in and recrystallized from ethanol. It had m.p. 216-218°. (Found: C, 44.5; H, 3.5; N, 15.6. $C_{24}H_{28}BrN_5O_3.2(C_6H_3N_3O_7)$ requires C, 44.5; H, 3.5; N, 15.8%).

EXAMPLE 10

4-(7'-Chloro-1',5'-naphthyridin-4'-ylamino)-2-diethylaminomethylphenol('5-azaamodiaquine') (TN 88)

4,7-Dichloro-1,5-naphthyridine (McCaustland, D.J., and Cheng, C.C. J.Heterocycl. Chem. 1970, 7, 467.) (0.2g), 4-amino-2- diethylaminomethylphenol dihydrochloride (0.27g) water (15.0ml) and methanol (5.0ml) were heated with stirring in an oil bath at 100° for 2h. The methanol was then evaporated under reduced pressure and the aqueous solution adjusted with ammonium hydroxide to pH 7-8. The yellow precipitate was collected, washed, dried, and recrystallized from cyclohexane to give 4-(7'-chloro-1',5'-naphthyridin-4'-ylamino)2-diethylaminomethyl-phenol (0.28g), m.p. 167-169°. (Found: C, 64.0; H, 6.0; N, 15.5. $C_{19}H_{21}ClN_4O$ requires C, 64.0; H, 5.9; N, 15.7%).

EXAMPLES 1 - 10

Toxicity Testing

The naphthyridines were tested for acute toxicity in mice by intraperitoneal injection in normal saline or peanut oil. Each test chemical was injected in a single dose of 200 mg/kg of body weight [except for

4-(7'-bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(diethylamino and pyrrolidin-1"-yl)methylphenol which, due to toxicity at 200 mg/kg, were run at 100 mg/kg] to three mice. No apparent ill effects were observed and all mice survived to and beyond 4 days in the above tests and in control experiments with normal saline and peanut oil.

Preliminary Antimalarial Screen

This was carried out as described previously. (Barlin, G.B., and Tan, E.-L., Aust.J.Chem., 1985, 38, 459; 1984, 37, 2469.) Each test chemical was given at a dosage of 200 mg/kg of body weight except for 4-(7'-bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(diethylamino and pyrrolidin-1"-yl)methylphenol which were at 100 mg/kg). The results are shown in Table 1.

TABLE 1:

Times given are those after injection of the chemical under test.
Time: h, hours; d, days; w, weeks; 0h denotes pretreatment.

Compound	Solvent[A]	Dose (mg/kg)	Mean percentage of parasite-infected red cells										
			0h	9h	24h	48h	3d	6d	8d	9d	14d	19d	4w
(1a)[J]	PO	200	16	34	62	88	B						
TN83	NS	200	27	30	9	<1	<1	11	8	4	<1	<1	<1
TN84	PO	200	16	27	16	3	5	26	14	5	<1	<1	<1
TN87	NS	200	19	26	3	<1	<1	25	28	18	<1	<1	<1
TN78	PO	200	18	24	5	<1	<1	<1	<1	<1	<1	<1	<1
TN77	NS	100	10	9	<1	<1	<1	<1	<1	<1	16	<1	<1
TN79	PO	200	22	32	7	<1	<1	<1	<1	<1	<1	<1	<1
TN80	PO	100	11	11	1	<1	<1	<1	<1	<1	<1	<1	<1
TN81	PO	200	17	13	1	<1	<1	<1	<1	<1	63	D	
TN82	PO	200	15	15	<1	<1	<1	<1	<1	<1	16	E	
TN88	PO	200	14	12	1	<1	<1	3	15	21	<1	<1	<1
NS	-	-	27	45	63	88	F						
PO	-	-	19	42	66	85	F						
Chloroquine[G]													
	NS	40	28	30	4	<1	<1	10	71	82[H]			

A PO, peanut oil; NS, normal saline.
B All three mice dead.
C Dihydrobromide.
D Two mice dead, parasitaemia of third mouse <1%.
E One mouse dead, parasitaemia of remaining two mice <1%.
F Two of the three mice dead at 3 days.
G Diphosphate.
H All mice dead at 10 days.
J 4-(7'-bromo-1',5'-naphthyridin-4'-ylamino)phenol.

EXAMPLE 11

2-Diethylaminomethyl-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (TN105)

4-Chloro-7-trifluoromethylquinoline (Snyder, H.Q., et al., J.Amer.Chem.Soc., 1947, 69, 371) (0.5g) and 4-amino-2-diethylaminomethylphenol dihydrochloride (0.75g) in a mixture of methanol (15.0ml) and water (5.0ml) were refluxed with stirring for 2h and the methanol evaporated under reduced pressure. After chilling, the yellow precipitate was collected, washed well with water, and recrystallized from aqueous ethanol to give 2-diethylaminomethyl-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (0.5g), m.p. 210-212°. (Found: C, 64.3; H.5.7; N.10.7. $C_{21}H_{22}F_3N_3O$ requires C, 64.8; H,5.7; N, 10.8%).

EXAMPLE 12

(a) 4-(7'-Trifluoromethylquinolin-4'-ylamino)phenol (12a)

4-Chloro-7-trifluoromethylquinoline (0.5g), p-aminophenol hydrochloride (0.41g), methanol (15ml) and water (5.0ml) were refluxed with stirring for 2h, and the methanol evaporated under reduced pressure. After chilling, the yellow precipitate was collected, suspended in water 30(ml), and adjusted with ammonium hydroxide to pH 7-8, and then recrystallized from aqueous ethanol to give 4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (0.6g), m.p. 253-254°. (Found: C, 61.3; H, 3.9; N, 8.9. $C_{16}H_{11}F_3N_2O.\frac{1}{2}H_2O$ requires C, 61.3; H, 3.9; N, 8.9%).

(b) 2,6-Bis(dimethylaminomethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (TN 107)

4'-(7'-Trifluoromethylquinolin-4'-ylamino)phenol (0.3g), formalin (5.0ml) and ethanolic dimethylamine (15.0ml, 33%) were refluxed with stirring for 20h. The solution was evaporated to dryness and the oily residue was subjected to t.l.c. (alumina; ether) to give an oil which slowly crystallized and was recrystallized from aqueous ethanol to give yellow crystals of 2,6-bis(dimethylaminomethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (0.32g), m.p. 86-88°. (Found, for a sample dried at 40°/0.2mmHg: C, 63.5; H, 6.2; N, 13.5. $C_{22}H_{25}F_3N_4O$ requires C, 63.2; H, 6.0; N, 13.4%).

EXAMPLE 13

2,6-Bis(diethylaminomethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (TN 108)

4-(7'-Trifluoromethylquinolin-4'-ylamino)phenol (0.3g), diethylamine (5.0ml) and formalin (5.0ml) in ethanol (10.0ml) were refluxed as above. The crude product was subjected to t.l.c. (alumina; chloroform) and recrystallized from a mixture of methanol and light petroleum (b.p. 60-80°) to give yellow crystals of 2,6-bis(diethylaminomethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (0.31g), m.p. 169-170°. (Found: C, 65.8; H, 7.1; N, 12.0. $C_{26}H_{33}F_3N_4O$ requires C, 65.8; H, 7.0; N, 11.8%).

EXAMPLE 14

2,6-Bis(dipropylaminomethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (TN 110)

4-(7'-Trifluoromethylquinolin-4'-ylamino)phenol (0.3g), dipropylamine (5.0ml), formalin (5.0ml) and ethanol (10.0ml) were refluxed as above. The crude product was subjected to t.l.c. (alumina, chloroform; then silica, ether) and the yellow oil crystallized on standing to give 2,6-bis(dipropylaminomethyl-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (0.20g), m.p. 113-114°. (Found, for sample dried at 80°/0.2mmHg: C, 67.9; H, 7.8; N, 10.5. $C_{30}H_{41}F_3N_4O$ requires C, 67.9; H, 7.8; N, 10.6%).

EXAMPLE 15

2,6-Bis(pyrrolidin-1"-ylamino)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (TN 109)

4-(7'-Trifluoromethylquinolin-4'-ylamino)phenol (0.3g), pyrrolidine (5.0ml), formalin (5.0ml) and ethanol (10.0ml) were refluxed as above, and excess pyrrolidine was removed by distillation in a vacuum. The residue was triturated with water (50ml), and the crude product subjected to t.l.c. (alumina, methanol; then alumina, ether) to give as a yellow oil, 2,6-bis(pyrrolidin-1"-ylmethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (0.23g). (Found: C, 64.1; H, 6.4; N, 10.9. $C_{26}H_{29}F_3N_4O.1.1H_2O$ requires C,63.7; H, 6.4; N, 11.4%).

EXAMPLE 16

2.6-Bis(piperidin-1"-ylmethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (TN 112)

4-(7'-Trifluoromethylquinolin-4'-ylamino)phenol (0.3g), piperidine (5.0ml), formalin (5.0ml) and ethanol (10.0ml) were treated as above. The crude product was purified by t.l.c. (alumina; chloroform) and recrystallized from cyclohexane to give 2,6-bis-(piperidin-1"-ylmethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (0.31g), m.p. 170-171°. (Found: C, 67.5; H, 6.9; N, 11.3. $C_{28}H_{33}F_3N_4O$ requires C, 67.5; H, 6.7; N, 11.2%).

EXAMPLE 17

2,6-Bis(morpholin-1"-ylmethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (TN 111).

4-(7'-Trifluoromethylquinolin-4'-ylamino)phenol (0.3g), morpholine (5.0ml), formalin (5.0ml) and ethanol (10.0ml) were treated as above to give, after t.l.c. (alumina; chloroform) and recrystallization from aqueous ethanol, yellow needles of 2,6-bis-(morpholin-1"-ylmethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (0.29g), m.p. 198-200°. (Found: C, 62.4; H, 5.9; N, 11.4. $C_{26}H_{29}F_3N_4O_3$ requires C, 62.1; H, 5.8; N, 11.15%).

EXAMPLES 10 - 17

Toxicity Testing

Each quinoline used in the antimalarial screening was tested for acute toxicity in three mice by intraperitoneal injection, each with a single dose in normal saline or peanut oil, at a dose of 100 mg/kg of body weight. No apparent ill effects were observed,

and all mice survived to and beyond 6 days in the above tests, and in control experim nts with normal saline and peanut oil.

Preliminary Antimalarial Screen

This was carried out as described previously. Each test chemical was given at a dosage of 100 mg/kg of body weight and blood counts were made at 9, 24, 48, and 72h and thereafter as shown in Table 2.

TABLE 2:

Times given are those after injection of the chemical under test.
Time: h, hours; d, days; w, weeks; 0h denotes pretreatment.

	Mean percentage of parasite-infected red cells													
A*	0h	9h	24h	48h	3d	6d	8d	9d	10d	11d	12d	14d	15d	4w
12a[H]	7	10	10	51	80	B								
TN105	11	5	<2	<1	<1	C	D	27	52	67	83	11	7	<1
TN107	6	3	1	<1[E]	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
TN108	6	14	6	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
TN110	11	6	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
TN109	8	4	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
TN112	5	2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
TN111	18	12	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
NS	25	35	65	86	[F]									
PO	20	23	58	85	[E]									
Chloroquine[G] (NS)	12	19	2	<1	<1									

A* Solvent - peanut oil (PO); dose of compounds in PO was 100 mg/kg. NS, normal saline.

B All mice dead in 4 days.

C Parasitaemia of one mouse 75%, other two mice <1%.

D One mouse dead, parasitaemia of other two 15%.

E One mouse dead.

F Mice dead at 3 days.

G Diphosphate: dose 40 mg/kg.

H 4-(7'-trifluoromethylquinolin-4'-ylamino)phenol.

EXAMPLE 18

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(4"-methylpiperazin-1"-ylmethyl)phenol (TN 117)

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)phenol (see Example 1(a)) (0.2g), formalin (2.0ml), N-methylpiperazine (2.0ml) and ethanol (10.0ml) were refluxed with stirring for 20h. Excess amine was distilled off under vacuum and the oily residue purified by t.l.c. (alumina; chloroform) to give a yellow oil (0.17g). (Found: C, 54.9; H, 6.2; N, 16.8. $C_{26}H_{34}BrN_7O.1.8H_2O$ requires C, 54.5; H, 6.6; N, 17.1%).

EXAMPLE 19

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(2"-methylpiperidin-1"-ylmethyl)phenol (TN 118).

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)phenol (0.2g), formalin (2.0ml), 2-methylpiperidine (2.0ml) and ethanol (10ml) were treated as above. Traces of 2-methylpiperidine were removed by triturating with water (50ml) before the oily residue was purified by t.l.c. (alumina; chloroform) to give a yellow oil (0.2g). (Found: C, 62.9; H, 6.9; N, 12.8. $C_{28}H_{36}BrN_5O$ requires C, 62.5; H, 6.7; N, 13.0%).

EXAMPLE 20

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(3",5"-dimethylpiperidin-1"-ylmethyl)phenol (TN 116).

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)phenol (0.2g), formalin (2.0ml), 3,5-dimethylpiperidine (2.0ml) and ethanol (10.0ml) were treated as above, and the oily residue purified on t.l.c. (alumina; chloroform, then silica; methanol) to give a yellow

oil (0.12g). (Found: C, 63.1; H, 7.2; N, 12.1. $C_{30}H_{40}BrN_5O$ requires C, 63.6; H, 7.1; N, 12.4%).

EXAMPLE 21

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2-(dimethylaminomethyl)-6-(pyrrolidin-1"-ylmethyl)phenol (TN 115)

4-(7'-Bromo-1',5'-naphthyridin-4'-ylamino)-2-(pyrrolidin-1"-ylmethyl)phenol (see Example 5). (0.15g), formalin (2.0ml) and ethanolic dimethylamine (10ml; 33%) were refluxed with stirring for 10h. The reaction mixture was evaporated to dryness in a vacuum to leave an oil which was purified by t.l.c. (alumina; chloroform) to give a yellow oil (0.11g). (Found: C, 57.5; H, 5.8; N, 14.9. $C_{22}H_{26}BrN_5O$ requires C, 57.9; H, 5.7; N, 15.3%).

EXAMPLE 22

(a) 4-(7'-Chloro-1',5'-naphthyridin-4'-ylamino)phenol

A mixture (c.1:1.5) of 4,7-dichloro-1,5-naphthyridine (McCaustland, D.J. and Cheng, C.C., supra) (0.66g), and p-aminophenol hydrochloride (0.72g), in methanol (20.0ml) and water (20.0ml) was refluxed with stirring for 2h. The methanol was then evaporated under reduced pressure and the aqueous solution adjusted to pH 8 with ammonium hydroxide. The yellow precipitate was collected, washed with water and recystallized from methanol to give 4-(7'-chloro-1',5'-naphthyridin- 4'-ylamino)phenol (0.7g), m.p. 240-241°. (Found: C, 61.9; H, 3.8; N, 15.1. $C_{14}H_{10}ClN_3O$ requires C, 61.9; H, 3.7; N, 15.5%).

(b) 4-(7'-Chloro-1',5'-naphthyridin-4'-ylamino)-2,6-bis(dimethylaminoethyl)phenol (TN 122)

4-(7'-Chloro-1',5'-naphthyridin-4'-ylamino)phenol (0.2g), formalin (2.0ml) and ethanolic dimethylamine (20.0ml; 33%) were treated as above to give a yellow oil (0.22g). (Found: C, 61.2; H, 6.4; N, 17.7. $C_{20}H_{24}ClN_5O.\frac{1}{2}H_2O$ requires C, 60.8; H, 6.4; N, 17.7%).

EXAMPLE 23

4-(7'-Chloro-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(diethylaminomethyl)phenol (TN 124)

4-(7'-Chloro-1',5'-naphthyridin-4'-ylamino)phenol (0.2g), formalin (2.0ml), diethylamine (2.0ml) and ethanol (10.0ml) were treated as above, and the residue purified by t.l.c. (silica; methanol) to give a yellow oil which slowly crystallized (0.19g), m.p. 106-107°. (Found: C, 65.0; H, 7.5; N, 15.7. $C_{24}H_{32}ClN_5O$ requires C, 65.2; H, 7.3; N, 15.8%).

EXAMPLE 24

4-(7'-Chloro-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(dipropylaminomethyl)phenol (TN 125)

4-(7'-Chloro-1',5'-naphthyridin-4'-ylamino)phenol (0.2g), formalin (2.0ml), dipropylamine (2.0ml) and ethanol (10.0ml) were refluxed with stirring for 40h (the reaction was incomplete at 20h). The solution was evaporated to dryness under reduced pressure and the residue purified on t.l.c. (silica; methanol) to give a yellow oil (0.20g). (Found: C, 66.4; H, 8.1; N, 13.8. $C_{28}H_{40}ClN_5O.\frac{1}{2}H_2O$ requires C, 66.3; H, 8.1; N, 13.8%).

EXAMPLE 25

4-(7'-Chloro-1',5'-naphthyridin-4'-ylamino)-2,6-bis-(pyrrolidin-1"-ylmethyl)phenol (TN 123).

A mixture of 4-(7'-chloro-1',5'-naphthyridin-4'-ylamino)phenol (0.2g), formalin (2.0ml), pyrrolidine (2.0ml) and ethanol (10.0ml) was treated as above, and the residue purified by t.l.c. (silica; methanol) to give a yellow oil (0.21g). (Found: C, 64.6; H, 6.6; N, 15.4. $C_{24}H_{28}ClN_5O.\frac{1}{2}H_2O$ requires C, 64.5; H, 6.5; N, 15.7%).

EXAMPLE 26

2,6-Bis(4"-methylpiperazin-1"-ylmethyl)4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (TN 121).

4-(7'-Trifluoromethylquinolin-4'-ylamino)phenol (see Example 12(a)) (0.2g), formalin (2.0ml), N-methylpiperazine (2.0ml) and ethanol (10.0ml) were treated as above. The product was purified by column (alumina; chloroform) and thin-layer chromatography (silica; methanol) to give a yellow oil which crystallized m.p. 163-164°. (Found: C, 62.9; H, 7.2; N, 15.7. $C_{28}H_{35}F_3N_6O.\frac{1}{2}H_2O$ requires C, 62.6; H, 6.8; N, 15.6%).

EXAMPLE 27

2,6-Bis(2"-methylpiperidin-1"-ylmethyl)-4'-(7'-trifluoromethylquinolin-4'-ylamino)phenol (TN 119)

A mixture of 4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (0.2g), formalin (2.0ml), 2-methylpiperidine (2.0ml) and ethanol (10.0ml) was treated as above. The oily residue was purified by t.l.c. (alumina; chloroform) to give a solid which was recrystallized from aqueous ethanol to give yellow

crystals (0.18g), m.p. 102-104°. (Found: C, 68.5; H, 7.3; N, 10.4. $C_{30}H_{37}F_3N_4O$ requires C, 68.4; H, 7.1; N, 10.6%).

EXAMPLE 28

2,6-Bis(3",5"-dimethylpiperidin-1"-ylmethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (TN 120)

4-(7'-Trifluoromethylquinolin-4'-ylamino)phenol (0.2g), formalin (2.0ml), 3,5-dimethylpiperidine (2.0ml) and ethanol (10.0ml) were treated as above. The product was purified by t.l.c. (alumina; chloroform) to give a yellow oil (0.19g). (Found: C, 67.0; H, 7.5; N, 9.7. $C_{32}H_{41}F_3N_4O.H_2O$ requires C, 67.1; H, 7.6; N, 9.8%).

EXAMPLES 18 - 28

Toxicity Testing

Each 1,5-naphthyridine and quinoline used for antimalarial screening was tested for acute toxicity in mice by intraperitoneal injection in normal saline or peanut oil. Each test chemical was injected in a single dose of 100 mg/kg of body weight [except for 2,6-bis(4"-methylpiperazin-1"-ylmethyl)-4-(7'-trifluoromethylquinolin -4'-ylamino)phenol which, due to the death of one mouse at a dose of 100 mg/kg, was run at 50 mg/kg] to three mice.

No apparent ill effects were observed, and all mice survived to and beyond 30 days in the above tests, and in control experiments with normal saline and peanut oil.

Preliminary Antimalarial Screen

This was carried out as described previously. Each test chemical was given at a dosage of 100 mg/kg

of body weight except for 2,6-bis(4"-methylpiperazin-1"-ylmethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol which was at 50 mg/kg, and blood counts were made at 9, 24, 48 and 72h and thereafter as shown in Table 3.

TABLE 3.

Times given are those after injection of the chemical under test.
Time: h, hours; d, days; 0h denotes pretreatment.

Mean percentage of parasite-infected red cells														
0h	9h	24h	48h	4d	4d	5d	6d	7d	8d	9d	10d	11d	12d	14d	19d
TN 117*															
10	4	1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	3	A	
TN 118*															
9	3	<1	<1	<1	<1	<1	<1	<1	<1	1	14	28	55	B	
TN 116*															
14	20	13	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
TN 115*															
9	6	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
TN 122*															
13	11	1	<1	<1	<1	<1	<1	<1	<1	<1	3	5	19	C	
TN 124*															
4	1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
TN 125*															
10	7	2	1	<1	<1	<1	2	6	17	21	27	7	3	2	<1 D
TN 123*															
9	5	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	E
TN 121**															
7	4	2	<1	<1	<1	<1	<1	<1	5	14	33	40	F		
TN 119*															
18	18	4	2	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	3	26	G
TN 120*															
12	12	6	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1
Normal saline															
11	19	28	57	H											
Peanut oil															
8	13	31	63	H											
Chloroquine[1] (normal saline - dose 40 mg/kg)															
24	18	3	<1	<1	<1	<1	2	2	<1	<1	<1	<1	<1	<1	<1

* Solvent used throughout (except where stated) - peanut oil; Dose 100 mg/kg.
** Dose 50 mg/kg.

A Parasitaemia of one mouse 12% at 14 days. This mouse dead at 19 days, parasitaemia of other two mice <1%.
B Two mice dead at 14 days, parasitaemia of remaining mouse 8% at 14 days and <1% at 19 days.
C One mouse dead at 14 days, parasitaemia of other two average 11%.
D Parasitaemia rose in one mouse only, to 80%, then declined.
E One mouse dead at 19 days, parasitaemia of other two mice <1%.
F Two mice dead at 12 days, parasitaemia of other mouse <1% at 19 days.
G All mice dead at 19 days.
H One mouse dead at 3 days, all mice dead at 4 days.
I Diphosphate.

EXAMPLE 29

Mono- and di-Mannich bases derived from 4-(7'-bromo or 7'-chloro-1',5'-naphthyridin-4'-ylamino)phenol and 4-(7'-trifluoromethylquinolin-4'-ylamino)phenol were assayed for antimalarial activity (using an in vitro radioisotopic technique) against three isolates of Plasmodium falciparum. The results are shown in Tables 4, 4A and 5. Many compounds from these two series had an IC_{50} value (concentration of compound causing 50% inhibition of ^{3}H-hypoxanthine incorporation) comparable to or better than those of mefloquine and amodiaquine, for both a chloroquine-sensitive isolate (FCQ-27) and a chloroquine-resistant isolate (K1). At least two compounds, 2,6-bis-(piperidin-1''-ylmethyl)-4-(7'-trifluormethylquinolin-4'-ylamino)phenol (TN112) and 2,6-bis(3",5"-dimethyl-piperidin-1"-ylmethyl)-4-(7'-trifluoromethylquinolin-4'-ylamino)phenol (TN1 120), showed significant superior activity to the three antimalarials against these isolates.

Some toxicity studies on the more active compounds have also been carried out and the results, together with those for chloroquine and amodiaquine for reference, are recorded in Table 6. The 1,5-naphthyridines TN78 and particularly TN79 were significantly less toxic than amodiaquine (or chloroquine). The 7-trifluoromethylquinolines TN108 and TN112 were, on the evidence available, also less toxic than amodiaquine and chloroquine.

Details of the materials and methods used in the testing against P.falciparum in vitro are set out below.

Parasites

The FCQ27 line of Plasmodium falciparum (origin: Papua New Guinea, first isolated at The Walter and Eliza Hall Institute of Medical Research, Melbourne) (Chen et al., Southeast Asian Journal of Tropical Medicine and Public Health, 1980, 11, 435), was obtained from Dr.G.Butcher (Royal Newcastle Hospital, Newcastle, Australia). The K1 line (origin: Kanchanaburi, Thailand) (Thaithong and Beale, Transactions of the Royal Society of Tropical Medicine and Hygiene, 1981, 75, 271) was obtained from Dr.A.Saul (Queensland Institute of Medical Research). The FCQ2 line (origin Madang, Papua New Guinea, first isolated at The Walter and Eliza Hall Institute of Medical Research, Melbourne) (Chen et al., supra) was obtained from Dr.A.Saul (Queensland Institute of Medical Research). All isolates were maintained routinely by the in vitro culture technique of Trager and Jensen (Science, 1976, 193, 673). Stock cultures contained a 5% suspension of washed human

erythrocytes, type O in RPMI 1640 medium (Flow), supplemented with 25mM HEPES-KOH pH 7.2, 32mM $NaHCO_3$ and 10% human serum type O. Gentamicin (40µg/ml) was added to all media. Cultures were maintained in modular incubator chambers (Flow) at 37.5°C in a gas mixture of 5% CO_2, 5% O_2 and 90% N_2.

Drugs

Chloroquine phosphate was purchased from Sterling Pharmaceuticals (Sydney, Australia) and amodiaquine hydrochloride from Parke Davis and Co., (Sydney). Mefloquine hydrochloride was a gift from the Walter Reed Army Institute of Research. The series of 4-(7'-bromo- and 7'-chloro-1',5'-naphthyridine-4'-ylamino)phenols and trifluoromethylquinolin-4'-ylamino phenols were prepared in the laboratories of the John Curtin School of Medical Research, Canberra. Stock solutions of chloroquine and amodiaquine were prepared in ddw and subsequently diluted in RPMI medium. Stock stolutions of mefloquine, and the naphthyridine and the trifluoromethylquinoline compounds were prepared in 10ml of 50% ethanol:ddw. Up to 4ml of alcohol was evaporated off under a current of air and the volume returned to 10ml with ddw. The solutions were subsequently diluted in ddw and thence in RPMI medium. Control experiments demonstrated that the remaining concentration of alcohol did not affect parasite growth and development.

Isotope

Incorporation of [G-^{3}H]-hypoxanthine was used as the index of parasite growth and development (Desjardins et al., Antimicrobial Agents and Chemotherapy, 1979, 16, 710). The isotope was

supplied as a lyophylate (5000 mCi/ml) in ampoules containing 5.0 mCi (Amersham, U.K.). For experimental purposes a solution containing 20µCi of ^{3}H-hypoxanthine per ml of 1640 medium was prepared.

Experimental

Microculture plates (Falcon, 96 well, flat bottom) were prepared as described by Desjardins et al (supra) with some modifications. A volume of 50µl of complete RPMI solution was first added to all wells. Then, leaving Row A as the control row a further 50µl of chloroquine in RPMI was added to the first three wells of Row B and serial twofold dilutions made across the plate and continued in Row C. The same procedure was repeated for the test compounds in subsequent rows. A working volume of 50µl was preferred to 25µl in order to reduce replicate error.

A constant volume (175µl) of a 1.5% haematocrit of unsynchronized parasitized erythrocytes (0.2-0.4% parasitaemia) was then added to all wells except the last three of Row A to which was added 175µl of a 1.5% haematocrit of unparasitized erythrocytes. Plates were then incubated for 24h at 37.5°C in the gas mixture described above. At this time a further 25µl of RPMI medium containing ^{3}H-hypoxanthine (0.1µCi) was added to all wells. The total volume was then 250µl giving a 1:10 dilution of the original drug concentration in the first three wells and twofold dilutions across the row. Determination of the IC_{50} (the concentration causing 50% inhibition of the incorporation of ^{3}H-hypoxanthine) was based on triplicate results for eight serial dilutions from a starting concentration of 64 nmol/l for all compounds

except chloroquine when the K1 isolate was being tested. Here the starting concentration was 1024 nmol/1.

Following the addition of the isotope, plates were incubated for a further 18h before the contents were harvested on a Titertek (Flow) semi-automated cell harvester and particulate matter deposited onto glass fibre discs. Discs were washed, dried, and placed into scintillation vials containing 5ml of 5% 2,5-diphenyloxazole (PPO) scintillant in toluene and radioactivity measured on an LKB 1217 Rack Beta liquid scintillation spectrometer.

Data analysis

Results were analysed using the technique described by Sixsmith et al (American Journal of Tropical Medicine and Hygiene, 1984, 33, 772). Computation of the concentration causing a 50% reduction in the incorporation of ^{3}H-hypoxanthine was calculated by interpolation using one data point above and one data point below the dpm midpoint between the parasitized and non-parasitized controls. A logarithmic transformation of both concentration and cpm values was made and the formula IC_{50} = antilog $(\log X_1 + [(\log Y_{50} - \log Y_1)\ (\log X_2 - \log X_1)/(\log Y_2 - \log Y_1)])$ was employed. Y_{50} was the dpm value midway between parasitized and non-parasitized control cultures and X_1, Y_1, X_2 and Y_2 are the concentration and dpm values for the data points above and below the dpm midpoints. Differences between mean IC_{50} values were compared using Student's t-test, with $P < 0.05$ considered significant.

Toxicity Testing

Mice were injected with a single i.p. dose of the test chemical in peanut oil (0.4ml) (except for amodiaquine hydrochloride and chloroquine diphosphate which were in 0.4ml normal saline), and were observed at 0.5h, 3h and daily thereafter to 7 days.

TABLE 4

In vitro antimalarial activity of a series of 4-(7'-bromo-1',5'-naphthyridin-4'-ylamino)phenols chloroquinine, amodiaquine and mefloquine against three isolates of P.falciparum

Inhibitor	50% inhibitory concentration (nmol/l)[A] P.falciparum strain		
	FCQ-27	K1	FCQ-2
TN63[E]	34.7 ± 11.0	>64	
TN77	4.6 ± 2.0	20.8 ± 8.9	4.3[D]
TN78	2.9 ± 1.1	6.6 ± 2.2	3.7[D]
TN79	2.5 ± 1.6	8.5 ± 4.0	8.1[D]
TN80	3.0 ± 0.9	9.3 ± 4.3	4.8[D]
TN81	34.8 ± 9.2	>64	
TN82	24.1 ± 0.3	>64	
TN83	28.8 ± 4.8	>64	
TN84	>64	>64	
TN87	34.7 ± 7.6	>64	
TN115	>64	>64	
TN116	1.3 ± 0.8	8.4 ± 6	0.5[D]
TN117	>64	>64	
TN118	-	22.6 ± 12	
Chloroquine	13.0 ± 6.4[B]	325 ± 199[B]	59.3 ± 20
Amodiaquine	2.9 ± 0.3[C]	11.7 ± 5.5[C]	2.6 ± 0.3
Mefloquine	10.1 ± 5.4	4.9 ± 3.3	24.6 ± 9

A Results are expressed as the mean (± SE) of three tests calculated after logarithmic transformation of data unless specified otherwise.

B Results are the mean of 10 tests.

C Results are the mean of 4 tests.

D Results from a single test.

E 4-(7'-bromo-1',5'-naphthyridin-4'-ylamino)-2-(diethylaminomethyl)phenol.

TABLE 4A

In vitro antimalarial activity of a series of 4-(7'-chloro-1',5'-naphthyridin-4'-ylamino)-phenols, chloroquine, amodiaquine and mefloquine against three isolates of P.falciparum

Inhibitor	50% inhibitory concentration (nmol/l)[A] P.falciparum strain		
	FCQ-27	K1	FCQ-2
TN122	-	16.2 ± 9.3	-
TN123	-	>64	-
TN124	2.9 ± 0.6	10.6 ± 2.2	-
TN125	1.8 ± 0.8[B]	16.7 + 5.3[B]	
Chloroquine	13.0 ± 6.4[C]	325 ± 199[C]	59.3 ± 20
Amodiaquine	2.9 ± 0.3	11.7 ± 5.5	2.6 ± 0.3
Mefloquine	10.1 ± 5.4	4.9 ± 3.3	24.6 ± 9

A Results are expressed as the mean (± SE) of three tests calculated after logarithmic transformation of data unless otherwise specified.

B Results are the mean of ten tests.

C Results are the mean of four tests.

TABLE 5

In vitro antimalarial activity of a series of 4-(7'-trifluoromethylquinolin-4'-ylamino)-phenols, chloroquine, amodiaquine and mefloquine against three isolates of P.falciparum

Inhibitor	50% inhibitory concentration (nmol/l)[A] P.falciparum strain		
	FCQ-27	K1	FCQ-2
TN105	2.1 ± 0.4	5.5 ± 2.7	
TN107	4.3 ± 1.8	7.0 ± 2.3	
TN108	1.0 ± 0.5	2.6 ± 0.3	1.7[E]
TN109	0.8 ± 0.4	2.1 ± 0.7	
TN110	1.7 ± 1.6	2.0 ± 0.7	
TN111	4.5 ± 0.4	6.8 ± 2.2	
TN112	0.5 ± 0.3[B]	0.8 ± 0.4[B]	0.7 ± 0.4[B]
TN119	0.4 ± 0.2[B]	6.6 ± 3.1[B]	
TN120	0.9 ± 0.6[B]	1.0 ± 0[B]	0.3[E]
TN121	3.1 ± 0.2[B]	8.6 ± 6[B]	
Chloroquine	13.0 ± 6.4[C]	325 ± 199[C]	59.3 ± 20
Amodiaquine	2.9 ± 0.3[D]	11.7 ± 5.5[D]	2.6 ± 0.3
Mefloquine	10.1 ± 5.4[B]	4.9 ± 3.3[B]	24.6 ± 9

A Results are expressed as the mean (± SE) of 5 tests calculated after logarithmic transformation of the data unless specified otherwise.

B Results are the mean of 3 tests.

C Results are the mean of 10 tests.

D Results are the mean of 4 tests.

E Result from a single test.

TABLE 6

Toxicity Testing

Compound	Dosage (mg/kg)	No.of mice in tests	No.of mice living at the following times after intra-peritoneal injection 0.5h	7d
TN78	200	6	6	6
TN79	800	3	3	3
TN80	100	6	6	6
TN108	200	6	5	5
TN112	200	6	5	5
	150	6	6	6
Amodiaquine*	200	6	1	1
	150	6	6	6
Chloroquine#	100	2	0	0

* Hydrochloride.

\# Diphosphate. The acute intraperitoneal LD_{50} of chloroquine in mouse is 73mg/kg.

EXAMPLE 30

In vivo evaluation of compounds TN77, TN112 and chloroquine against the "ANKA" strain of P.bergei in mice was carried out in a single test as detailed below.

Parasites

The Antwerp/Kasapa (ANKA) strain of Plasmodium bergei was isolated on 7 March, 1965 at the Prince Leopold Institute, Antwerp, Belgium from infected mosquitoes caught in the forest galleries of Kasapa, Zaire by Bafort [Vincke,I.H. and Bafort,J. (1968). Resultats de deux ans d'observation sur la transmission cyclique de Plasmodium berghei. Ann.Soc.Belg.Med.Trop., 48, 439-454]. This strain was obtained from the School of Public Health and Tropical Medicine, Sydney, Australia.

Protocol

The test mice were each given a dose of 10 million parasites of the ANKA strain of P.berghei in sodium citrate on Day 1. The parasitaemia of the mice was then allowed to reach c 20% on Day 5. Then a dose 0.8mg/kg of TN77, TN112, and chloroquine were each given orally to three separate replicate mice for four consecutive days and the parasitaemia followed for up to 21 days.

The results are set out in Table 7.

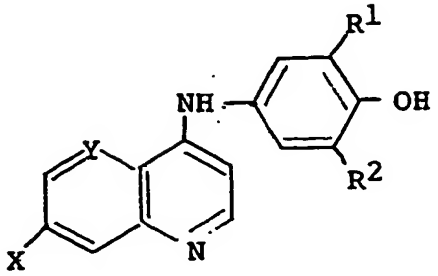
TABLE 7

Preliminary antimalarial screening results against ANKA strain of P.berghei in mice. Results are the average for three mice with the drugs given on Days 5, 6, 7 and 8.

Drug	Day 5	Day 7	Day 9	Day 11	Day 13	Day 21
Nil (control)	22	30	48	55	All dead	
TN77	24	0	0	0	0	0
TN112	27	0	0	0	0	0
Chloroquine	21	13	25	49	50	All dead

CLAIMS:

1. A compound of the general formula (I):



I

wherein X represents a halogen or halogen-substituted alkyl group;
Y represents CH or N; and
R^1 and R^2, which may be the same or different, represent hydrogen, aminoalkyl, mono- or di-alkylaminoalkyl, mono- or di-(substituted alkyl)aminoalkyl, mono- or di-(cycloalkyl)aminoalkyl, mono- or di-(aryl)aminoalkyl, mono- or di-(substituted aryl)aminoalkyl, or alkyl substituted by a nitrogen-containing heterocyclic or mono- or di-alkyl-substituted heterocyclic group;

provided that R^1 and R^2 are not both hydrogen; and that R^2 is not diethylaminomethyl when X is chloro, Y is CH and R^1 is hydrogen; or when X is bromo, Y is N and R^1 is hydrogen.

2. A compound according to claim 1, wherein X represents halogen and Y represents N.

3. A compound according to claim 1, wherein X represents a halogen-substituted alkyl group and Y represents CH.

4. A compound according to claim 1 wherein X represents a bromo, chloro or fluoro group, or a trifluoromethyl group.

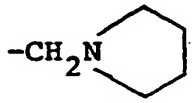
5. A compound according to claim 1, wherein R^1 and/or R^2 represent aminomethyl, mono- or di-alkylaminomethyl, mono- or di-(substituted alkyl)aminomethyl, mono- or di-(cycloalkyl)aminomethyl, mono- or di-(aryl)aminomethyl, mono- or di-(substituted aryl)aminomethyl, or methyl substituted by a nitrogen-containing heterocyclic or mono- or di-alkyl substituted heterocyclic group.

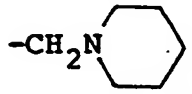
6. A compound according to claim 1, wherein R^1 and/or R^2 represent:

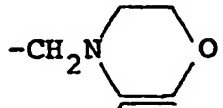
$-CH_2NMe_2$

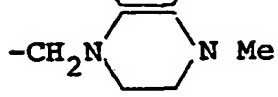
$-CH_2NEt_2$

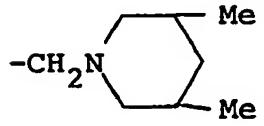
$-CH_2NPr_2$

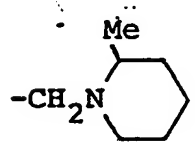






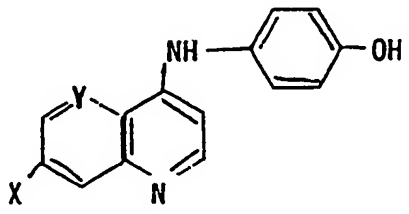






7. A compound according to claim 1, wherein both R^1 and R^2 are other than hydrogen.

8. A process for the preparation of a compound of the general formula (I), as defined in claim 1, which comprises reaction of a compound of the general formula (II):

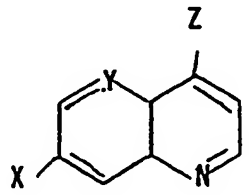


II

in which X and Y are as defined in claim 1, with an appropriate amine or nitrogen-containing heterocyclic compound in the presence of formaldehyde.

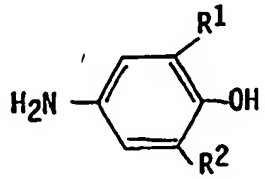
9. A process according to claim 8, wherein the compound of general formula II is refluxed with the appropriate amine or heterocyclic compound in ethanolic formalin.

10. A process for the preparation of a compound of the general formula (I) as defined in claim 1 which comprises reaction of a compound of general formula III:



III

in which X and Y are as defined in claim 1, and Z represents halogen, with an aminophenol of the general formula IV:



IV

in which R^1 and R^2 are as defined in claim 1.

11. A process according to claim 10, wherein the reaction is performed under reflux in aqueous methanol.

12. A pharmaceutical composition for use in treatment of malaria in an animal, including a human, which comprises an effective amount of a compound of the general formula (I) as defined in claim 1, together with a pharmaceutically acceptable carrier or diluent therefor.

13. A method of treating malaria in an animal, including a human, which comprises administering to the animal an effective amount of a compound of the general formula (I) as defined in claim 1.

INTERNATIONAL SEARCH REPORT

International Application No PCT/AU 86/00142

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl.[4] C07D 215/46, 471/04, A61K 31/44, 31/47

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	C07D 215/46, 471/04
US Cl.	546/122, 546/123, 546/162

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

AU : IPC as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	US,A, 4421920 (BAUDOUIN et al) 20 December 1983 (20.12.83)	(1,3-13)
X	AU,A, 18299/83 (AMERICAN HOSPITAL SUPPLY CORP.) 23 February 1984 (23.02.84)	(1,3-12)
X	DE,A, 1817577 (FRIEDHEIM) 16 October 1969 (16.10.69)	(1,3-7)
A	FR,A, 1437359 (PARKE, DAVIS & CO.) 28 March 1966 (28.03.66)	
A	AU,B, 537068 (59799/80) (WARNER-LAMBERT CO.) 7 June 1984 (07.06.84)	
A	US,A, 3948920 (NABIH) 6 April 1976 (06.04.76)	
A	Australian Journal of Chemistry, Volume 37, issued 1984, G.B. Barlin et al, "Potential Antimalarials. I 1,8-Napthyridines", see pages 1065-73.	
A	Australian Journal of Chemistry, Volume 37, issued 1984, G.B. Barlin et al, "Potential Antimalarials. II N4-Substituted 2-Methoxy (and 2-Hydroxy)-1,5-Napthyridin-4-amines", see pages 2469-77.	

CONTINUED

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
5 September 1986 (05.09.86)	17 SEP 1986
International Searching Authority	**Signature of Authorized Officer**
Australian Patent Office	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Australian Journal of Chemistry, Volume 38, issued 1985, G.B. Barlin et al, "Potential Antimalarials. III N^4-Substituted 7-Bromo-1,5-Napthyridin-4-amines", see pages 459-65.	
P,X	Australian Journal of Chemistry, Volume 38, issued 1985 June, G.B. Barlin et al, "Potential Antimalarials. IV 4-[7'-Bromo (and Chloro)-1',5'-napthyridin-4'yl amino]phenols and N^4-Substituted 7-Chloro-1,5-napthyridin-4-amines", see pages 905-11.	(1,2,4-13)
A	Journal of the American Chemical Society, Volume 69, issued 1947, H.R. Snyder et al, "Synthesis of 4-Hydroxyquinolines VIII. Some Halogen Containing 4-Aminoquinoline Derivatives", see pages 371-4.	
X	Journal of Medicinal Chemistry, Volume 24, issued 1981, Mei-lin Go et al, "Synthesis of some novel Amodiaquine Analogues as Potential Antimalarial and Antifilarial Compounds", see pages 1471-5.	(1,3-13)
X	Life Sciences, Volume 36, issued 1985, F.C. Churchill et al, "Amodiaquine as a Prodrug: Importance of Metabolite(s) in the Antimalarial Effect of Amodiaquine in Humans", see pages 53-62.	(1,3-7,13)
X	Annals of Tropical Medicine and Parasitology, Volume 76, No.3, issued 1982, D.C. Warhurst et al, "The chemotherapy of rodent malaria XXXIII", see pages 257-64.	(1,3-7,12-13)
X	Annals of Tropical Medicine and Parasitology, Volume 74, No.3, issued 1980, W. Peters et al, "The experimental chemotherapy of leishmamiasis, VII", see pages 321-35.	(1,3-7,12-13)
X	Southeast Asian Journal of Tropical Medicine and Public Health, Volume 12, No.1, issued 1981 March, M.L. Go et al, "Investigation of the Anti-Acetylcholinesterase Activities of the Antimalarial agent, Amodiaquine, and related compounds", see pages 37-41.	(1,3-7,12)
X	Biochemical Pharmacology, Volume 18, No.8, issued 1969, L.D. Zeleznick et al, "Immunosuppression by compounds which complex with deoxyribonucleic acid", see pages 1823-7.	(1,3-7,12-13)
X	The American Journal of Tropical Medicine and Hygiene, Vol 16, No.2, issued 1967, P.E. Thompson et al, "Relations among antimalarial drugs: Results of studies with Cycloguanil-, Sulfone-, or Chloroquine-resistant Plasmodium Berghei in mice", see pages 133-45.	(1,3-7,12-13)

CONTINUED

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category*	Citation of Document with indication where appropriate, of the relevant passages	Relevant to Claim No
X	Chemical Abstracts, Volume 99, No.7, issued 1983, August 15 (Columbus, Ohio, U.S.A.), Ren Daoxing et al, "Synthesis, toxicity and antimalarial effects of bispyroquine", see page 542, column 1, the abstract no. 53568s.	(1,3-13)
X	Chemical Abstracts, Volume 93, No.7, issued 1980, August 18 (Columbus, Ohio, U.S.A.), Huang Lan-Sun et al, "Synthesis of M6407, a new antimalarial agent", see page 952, columns 1-2, the abstract no. 71499a.	1,3-13)
X	Chemical Abstracts, Volume 96, No.17, issued 1982, April 26 (Columbus, Ohio, U.S.A.), Li Fulin et al, "Studies on antimalarials - synthesis of 4-(arylamino)-2-[tert-butylamino)methyl] phenols", see page 748, column 1, the abstract No. 142733x.	(1,3-13)
X	Chemical Abstracts, Volume 59 (Columbus, Ohio, U.S.A.), W.L. Nobles et al, "Antimalarial agents VIII. Synthesis of amopyroquine", see abstract No. 8700g.	(1,3-13)
X	Chemical Abstracts, Volume 54 (Columbus, Ohio, U.S.A.), V.F. Gladkikh et al, "Some data on the tolerance of laboratory animals to Cycloquine - an antimalarial preparation", see abstract no. 6956g.	(1,3-7, 12-13)
X	Yaoxue Xuebao, Volume 19, No.11, issued 1984, Shen Jihua et al, "Studies on antimalarial agents. XIV. Synthesis of trifluoromethylquinolyltetrahydro-napthols and -aminophenols", pages 856-9. (Chemical Abstracts, Volume 102, No.21, issued 1985 May 27 (Columbus, Ohio, U.S.A.) see page 568, column 1, abstract no. 184957w.)	(1,3-13)
X	Journal of Medicinal Chemistry, Volume 12, No.4, issued 1969, E.F. Elslager et al, "Repository drugs. VIII. Ester and amide congerers of amodiaquine, hydroxychloroquine, oxychloroquine, primaquine, quinacrine, and related substances as potential long acting antimalarial agents", see pages 600-7 (Chemical Abstracts Registry No. [21740-09-0]).	

FURTHER INFORMATION C NTINUED FROM THE SECOND SHEET

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 13 because they relate to subject matter not required to be searched by this Authority, namely:

Methods for treatment of the human or animal body by therapy.

2. [ ] Claim numbers ....., because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

176+153/2

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON
INTERNATIONAL APPLICATION NO. PCT/AU 86/00142

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Members
DE 1817577	CH 536841
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